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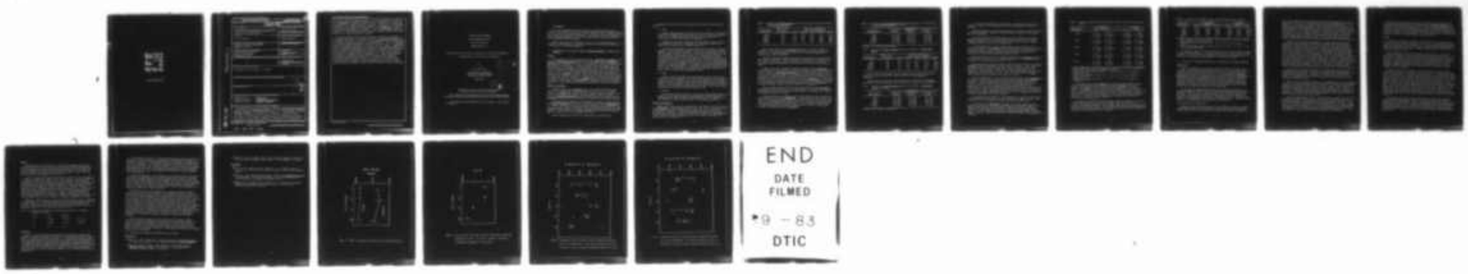

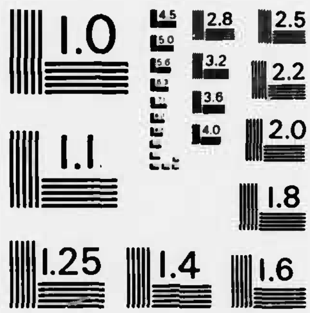


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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The pathogenicity of the entomogenous nematodes, Neoaplectana carpocapsae (DD-136 strain) and Neoaplectana glaseri, was tested for the German cockroach, Blattella germanica, under laboratory conditions. All developmental stages, including oothecae, were tested for susceptibility. Infection dishes consisted of standard size petri dishes lined with filter paper, to which were added 3 ml of a nematode suspension containing infective juveniles at a concentration of 1000, 5000, 10,000, and 20,000 per ml. The dishes were examined for cockroach mortality at 24 hour intervals for a period of 5 days.

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Both German cockroach adults and nymphs were very susceptible to infection by N. carpocapsae. Significant mortality was recorded after 3 days for all concentrations of infective juveniles. Higher concentrations of infectives resulted in a more rapid increase in mortality. N. glaseri was significantly less pathogenic for German cockroach adults than N. carpocapsae. Nymphal instars were unsusceptible to infection. In both cases, reproduction of the nematodes in cockroach cadavers was negligible. Neither nematode could penetrate the hard outer shell of the oothecae.

Data were gathered to test three hypotheses concerning the function of the aggregation behavior of the German cockroach, (Blattella germanica (L.) (Orthoptera: Blattidae). The first is that aggregation serves to ameliorate in a harsh environment and promote individual survival. Group and individual rearing experiments demonstrated a possible reduction of survival and growth rates with increased group size, in contrast with the increases suggested in previous literature. Bioassays demonstrated that information concerning the sexual composition of a population is present in residues left by the population, and that this information may be exploited by other individuals. This result in consistent with two hypotheses, i.e. that roaches aggregate for purposes of mating, and that they might select habitats based on information available from conspecifics. Bioassays also showed that a natural population of roaches, which had been subject to mortality due to control efforts, displayed a stronger aggregation response than did the laboratory strain, a result consistent with the habitat selection hypothesis.

OFFICE OF NAVAL RESEARCH

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ANNUAL REPORT NO. 3

# A New Approach for the Control of Cockroaches Utilizing the Entomophilic Nematode DD-136 in Conjunction with Attractants

by

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August 1983

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## I. INTRODUCTION

This report represents the third and final year research results on this contract. The original objectives were aimed at developing a pest management scheme for the control of cockroaches using a virulent nematode (DD-136), in conjunction with baits, attractants and innoculation devices. The research has since been modified to also include the possible use of juvenile hormone analogs in cockroach control, as well as cockroach behavioral studies.

Progress this year can be viewed as two distinct, yet interlocking areas of research: a) susceptibility of the German cockroach to neoaplectanid nematodes, and b) the function of cockroach aggregation.

## II. SUSCEPTIBILITY OF THE GERMAN COCKROACH, *BLATTELLA GERMANICA*, TO NEOAPLECTANID NEMATODES.


### Abstract

The pathogenicity of the entomogenous nematodes, *Neoaplectana carpocapsae* (DD-136 strain) and *Neoaplectana glaseri*, was tested for the German cockroach, *Blattella germanica*, under laboratory conditions. All developmental stages, including oothecae, were tested for susceptibility. Infection dishes consisted of standard size petri dishes lined with filter paper, to which were added 3 ml of a nematode suspension containing infective juveniles at a concentration of 1000, 5000, 10,000, and 20,000 per ml. The dishes were examined for cockroach mortality at 24 hour intervals for a period of 5 days. Both German cockroach adults and nymphs were very susceptible to infection by *N. carpocapsae*. Significant mortality was recorded after 3 days for all concentrations of infective juveniles. Higher concentrations of infectives resulted in a more rapid increase in mortality. *N. glaseri* was significantly less pathogenic for German cockroach adults than *N. carpocapsae*. Nymphal instars were unsusceptible to infection. In both cases, reproduction of the nematodes in cockroach cadavers was negligible. Neither nematode could penetrate the hard outer shell of the oothecae.


### Materials and Methods

The DD-136 strain of *N. carpocapsae* used in this study was provided by Harry Kaya, Associate Nematologist at the University of California, Davis. Original cultures of *N. glaseri* were furnished by James Lindegren, research entomologist with the U.S.D.A. Stored-Products Insects Research Laboratory, Fresno, California. A culture of *Xenorhabdus nematophilus*, the bacterium symbiotically associated with *N. carpocapsae*, was provided by G. M. Thomas of the University of California, Berkeley.

Both nematodes were propagated in the laboratory on larvae of the greater wax moth, *Galleria mellonella*, and on adults of the grey house cricket, *Acheta domestica* L. Infective juveniles were stored in an aqueous suspension of 0.15% formalin at 7°C. Prior to use, invasives were washed three times with distilled water and passed through nylon mesh filters to ensure that the final suspension contained only viable juveniles. The concentration of nematode suspensions was estimated by counting the number of invasives in 1 ml of an appropriate dilution.

German cockroaches were reared in the laboratory on Purina® dog chow.

Experimental infections with both nematodes were performed in the following manner.

1. Adults

Infection dishes consisted of plastic petri plates (100 x 15 mm) lined with three sheets of Whatman's #1 filter paper (7.0 cm). Each dish was inoculated with 3 ml of a nematode suspension containing infective juveniles at a concentration of 1000, 5000, 10,000, or 20,000 per ml.

Approximately 200 adult cockroaches (excluding gravid females), divided evenly among ten replicates, were exposed to each nematode concentration. Control plates received 3 ml of distilled water each.

The dishes were examined at 24-hour intervals for five days for cockroach mortality. Many of the dead cockroaches were dissected and the body contents examined for the presence of nematodes. In addition, the hemolymph of several dead cockroaches was sampled for the presence of the symbiotic bacterium. This was accomplished in the following manner: test cockroaches were surface sterilized in 0.4% Hyamine 10-X for 5 minutes, and then washed three times in sterile distilled water. After being transferred to sterile dissection dishes, the antennae of the cockroaches were cut off close to the base. Hemolymph was collected in sterile micropippettes from the ends of the cut antennae by gently prodding the cockroach's abdomen. The hemolymph was streaked onto nutrient agar plates, or into AC broth medium. All bacterial isolates were incubated in the dark at 28°C. The remaining dead cockroaches were "trapped", and held for 14 days, in order to collect any new emerging juveniles.

2. Nymphs

Cockroach nymphs were divided into three groups in order to test instar susceptibility to the nematodes. The first group consisted of first and second instars, the second consisted of third and fourth instars, and the last group consisted of fifth and sixth instars. Twenty representatives of each group were exposed to nematode suspensions containing various concentrations of infective juveniles in the same manner as described for the adults. The infection dishes were examined for 3 days for cockroach nymph mortality.

3. Oothecae

Fully developed oothecae (24-30 d. old) were removed from gravid females and placed in typical infection dishes (10 oothecae/dish) contailing nematode concentrations of 5000, 10,000 and 20,000 juveniles per dish. After 72 hours, all oothecae were dissected and examined for the presence of nematodes.

## Results and Discussion

German cockroach adults were very susceptible to infection by the DD-136 strain of *N. carpocapsae*. Average mortality rates of 52.0, 91.7, 94.3, and 98.0 percent were recorded after 72 hours for concentrations of 1000, 5000, 10,000, and 20,000 infectives/ml, respectively. Control plates showed an average of 11.0% mortality in the same period. Mortality increased to 100.0% after 120 hours for concentrations of 5000 infectives/ml and above, although mortality inthe controls increased to an average of nearly 40.0% (Table 1).

TABLE 1. Mortality of *Blattella germanica* adults after exposure to the DD-136 strain of *Neoaplectana carpocapsae*.

| Concentrations of infectives/ml | Number of test cockroaches (in 10 replicates) | Cumulative mortality (%) (avg. for 10 replicates) | | | | |
|---|---|---|---|---|---|---|
| | | 24 hr | 48 hr | 72 hr | 96 hr | 120 hr |
| 1,000 | 194 | 4.1 | 26.2 | 52.0 | 74.5 | 83.7 |
| 5,000 | 195 | 18.5 | 67.6 | 91.7 | 97.9 | 100.0 |
| 10,000 | 194 | 17.4 | 77.2 | 94.3 | 99.0 | 100.0 |
| 20,000 | 187 | 37.9 | 90.1 | 98.0 | 100.0 | 100.0 |
| Control | 213 | 0.1 | 4.5 | 11.0 | 22.3 | 39.5 |

Higher concentrations of *N. carpocapsae* resulted in a more rapid increase in mortality. Thus, the 50% mortality level was reached 29 hours after exposure to a concentration of 20,000 infectives/ml compared to 71 hours after exposure to a concentration of 1000 infectives/ml.

Infected cockroaches displayed uncharacteristic behavior before death, moving about slowly and trembling. Third-stage juveniles could readily be observed through the integument of dead cockroaches, especially in the head capsule and abdomen. Dissections revealed adult nematodes in the hemocoel as soon as 48 hours after host death.

Attempts to isolate the symbiotic bacterium associated with *N. carpocapsae* fromthe hemolymph of dead cockroaches consistently yielded a gram-negative, rod-shaped bacterium similar to *Xenorhabdus nematophilus* in morphology and colony characteristics. Other bacteria, presumably gut contaminants, were sometimes found. Although a comprehensive characterization of the suspected symbiote was not undertaken, the reactions to a number of selected biochemical tests were determined. The results were compared with the reactions of a known pure culture of *X. nematophilus*. On the basis of these tests, and the published description of the symbiote, the bacterium isolated from the hemolymph of infected cockroaches was almost certainly *X. nematophilus*. The hemolymph of healthy control cockroaches contained no microorganisms.

German cockroach nymphs were also susceptible to infection by *N. carpocapsae*. High mortality rates were recorded for all three groups of instars after 72 hours at concentrations of 5000 infectives/ml and above (Table 2). No mortality was recorded in the control plates after 3 days.

Infective juveniles of *N. glaseri* proved to be significantly less pathogenic for German cockroach adults than *N. carpocapsae*. Average mortality rates of 10.0, 29.1, 22.0, and 23.2 percent were recorded after 72 hours for concentrations of 1000, 5000, 10,000, and 20,000 infectives/ml, respectively. After 120 hours, the control plates actually showed slightly more mortality than any of the nematode treatments (Table 3). Higher concentrations of infective juveniles did not result in a more rapid increase in mortality, as with *N. carpocapsae*.

TABLE 2. Mortality of *Blattella germanica* nymphs after exposure to the DD-136 strain of *Neoaplectana carpocapsae* for 72 hours.

| Concentration of infectives/ml | Mortality (%) 1st and 2nd instars | 3rd and 4th instars | 5th and 6th instars |
|---|---|---|---|
| 1,000 | 45.5 (22)[a] | 53.8 (13) | 40.0 (20) |
| 5,000 | 77.8 (18) | 64.7 (17) | 54.5 (22) |
| 10,000 | 89.5 (19) | 85.0 (20) | 60.0 (15) |
| 20,000 | 93.3 (15) | 100.0 (17) | 94.4 (18) |
| Control | 0.0 (20) | 0.0 (20) | 0.0 (20) |

[a] Number of test nymphs in parentheses.

TABLE 3. Mortality of *Blattella germanica* adults after exposure to *Neoaplectana glaseri*

| Concentrations of infectives/ml | Number of test cockroaches (in 10 replicates) | Cumulative mortality (%) (avg. for 10 replicates) 24 hr | 48 hr | 72 hr | 96 hr | 120 hr |
|---|---|---|---|---|---|---|
| 1,000 | 200 | 2.0 | 7.0 | 10.0 | 16.0 | 25.5 |
| 5,000 | 189 | 8.7 | 18.3 | 29.1 | 35.0 | 38.0 |
| 10,000 | 178 | 5.3 | 14.4 | 22.0 | 25.4 | 28.8 |
| 20,000 | 197 | 8.3 | 15.6 | 23.2 | 32.8 | 35.8 |
| Control | 213 | 0.1 | 4.5 | 11.0 | 22.3 | 39.5 |

Although third-stage juveniles could be observed in some of the roach cadavers, attempts to isolate the symbiotic bacterium associated with *N. glaseri* were unsuccessful.

German cockroach nymphs were rather unsusceptible to infection by *N. glaseri*. Low mortality rates were recorded after 72 hours for all groups of instars (Table 4).

TABLE 4. Mortality of *Blattella germanica* nymphs after exposure to *Neoaplectana glaseri* for 72 hours.

| Concentration of infectives/ml | Mortality (%) 1st and 2nd instars | 3rd and 4th instars | 5th and 6th instars |
|---|---|---|---|
| 1,000 | 10.0 (20)[a] | 10.0 (20) | 15.7 (19) |
| 5,000 | 10.0 (20) | 0.0 (20) | 30.0 (20) |
| 10,000 | 10.0 (20) | 10.5 (19) | 29.4 (17) |
| 20,000 | 15.0 (20) | 25.0 (20) | 25.0 (20) |
| Control | 0.0 (20) | 0.0 (20) | 0.0 (20) |

[a] Number of test nymphs in parentheses.

In all cases, reproduction of the nematodes in the cockroach cadavers was very limited. The number of second-generation infectives emerging after 14 days was negligible.

Third-stage juveniles of either nematode could not be observed upon dissection of any cockroach oothecae. Apparently, they were unable to penetrate the hard outer shell of the egg cases.

In order to facilitate statistical inference about the means of the different nematode treatments, an arc sine transformation of the mortality data for both nematodes was performed. The values of the transformed data for adult and nymph cockroaches appear in Tables 5 and 6, respectively.

For adult cockroaches, the amount of mortality resulting from each of the nematode treatments of N. carpocapsae differed significantly from the control at both 3 days and 5 days ($p < .005$). By the LSD test (5% level), the treatment of 1000 infectives/ml differed significantly than treatments of 5000, 10,000, 20,000 infectives/ml did not differ significantly from each other.

For German cockroach nymphs, all nematode treatments of N. carpocapsae resulted in significant mortality ($p < .005$). The different groups of instars were equally susceptible to a given nematode treatment ($p > .10$).

The amount of adult cockroach mortality resulting from the nematode treatments of N. glaseri differed significantly from the control of 3 days ($p < .005$). By the LSD LSD test (5% level), the treatments of 5000, 10,000, and 20,000 did not differ significantly from each other, although they did differ significantly from the treatment of 1000 infective/ml. However, after 5 days, the treatments did not differ significantly from the control ($p > .10$). None of the treatments of N. glaseri against nymphs resulted in significant mortality ($p > .05$).

From this investigation, it is clear that the DD-136 strain of N. carpocapsae has the ability to infect and kill German cockroach adults and nymphs under laboratory conditions. Although the experimental methods provided optimum conditions for infection, limiting the mobility of the cockroaches, and assuring host-parasite contact, the ability of the third-stage juveniles to induce mortality was significant. A concentration of 5000 infectives/ml was sufficient to result in 91.7% mortality after 3 days. The higher concentrations provided more rapid knockdown, but did not result in significantly higher total mortality after 3 days. This indicates that there is an optimum application rate per area, and that exceeding it would be an unnecessary expenditure of infective juveniles.

The unexpectedly high mortality in the controls after 5 days was probably due to a lack of food. For this reason, it is more appropriate to compare the 3 day totals of mortality, when the mortality observed in the control was only 11.0%.

The inability of N. carpocapsae to reproduce in the roach cadavers is both puzzling and unfortunate. The results indicated that the symbiotic bacterium could establish itself in the cockroach cadavers, supposedly creating the necessary conditions for development and reproduction. Although the third-stage juveniles matured to the adult stage, they were unable to produce new generations of infective stages. Apparently, some other factors were limiting the ability of the nematodes to reproduce.

TABLE 5. Values of transformed mortality data for German cockroach adults, after 3d. and 5d.

| Concentration of infectives/ml | *N. carpocapsae* 3d. | *N. carpocapsae* 5d. | *N. glaseri* 3d. | *N. glaseri* 5d. |
|---|---|---|---|---|
| 1,000 | n = 194<br>$\bar{x}$ = .557*<br>s = .180 | n = 194<br>$\bar{x}$ = 1.029**<br>s = .225 | n = 200<br>$\bar{x}$ = 0.090+<br>s = .062 | n = 200<br>$\bar{x}$ = .259++<br>s = .106 |
| 5,000 | n = 195<br>$\bar{x}$ = 1.211*<br>s = .207 | n = 195<br>$\bar{x}$ = 1.571**<br>s = 0.0 | n = 189<br>$\bar{x}$ = .297+<br>s = .103 | n = 189<br>$\bar{x}$ = .391++<br>s = .097 |
| 10,000 | n = 194<br>$\bar{x}$ = 1.312*<br>s = .232 | n = 194<br>$\bar{x}$ = 1.571**<br>s = 0.0 | n = 178<br>$\bar{x}$ = .226+<br>s = .177 | n = 178<br>$\bar{x}$ = .304++<br>s = .246 |
| 20,000 | n = 187<br>$\bar{x}$ = 1.461*<br>s = .180 | n = 187<br>$\bar{x}$ = 1.571**<br>s = 0.0 | n = 197<br>$\bar{x}$ = .236+<br>s = .109 | n = 197<br>$\bar{x}$ = .368++<br>s = .095 |
| Control | n = 213<br>$\bar{x}$ = .111<br>s = .077 | n = 213<br>$\bar{x}$ = .409<br>s = .115 | n = 213<br>$\bar{x}$ = .111<br>s = .077 | n = 197<br>$\bar{x}$ = .408<br>s = .115 |

* Differ significantly from the control at $p < .005$; $F = 98.49$ on (4,45) degrees of freedom; $R^2 = 89.74$ (percent of explained variation).

** Differ significantly from control at $p < .005$; $F = 207.94$ on (4,45) degrees of freedom; $R^2 = 94.86$.

\+ Differ significantly from control at $p < .005$; $F = 6.13$ on (4,45) degrees of freedom; $R^2 = 35.25$.

++ Differ significantly from control at $p < .10$; $F = 1.90$ on (4,45) degrees of freedom; $R^2 = 14.4$.

Third-stage juveniles of N. glaseri are significantly less pathogenic for all stages of the German cockroach than infectives of N. carpocapsae. The relative inability of N. glaseri to induce cockroach mortality could be due to several factors. the larger size of the invasives (1.06 mm compared to .547 mm for DD-136; could be a disadvantage, making it more difficult to enter natural body openings. The greater surface area could render them more susceptible to dessication. This may account for the inability of the infectives to cause significant cockroach mortality after 3 days. The infectives of N. glaseri may be less active than the DD-136 invasives when seeking a new host.

The potential for application of entomogenous nematodes in practical situatios to control cockroach populations is probably limited. Their susceptibility to dessication precludes the potential use of aqueous sprays that could be applied easily to areas of cockroach infestation. The development of additives that would extend the survival time of infective stages in the field might eventually increase the number of situations in which nematodes could be employed.

TABLE 6. Values of the transformed mortality data for German cockroach nymphs.

| Concentration of infectives/ml | *N. carpocapsae** | | | *N. glaseri*** | | |
|---|---|---|---|---|---|---|
| | Group 1[a] | Group 2 | Group 3 | Group 1 | Group 2 | Group 3 |
| 1,000 | 0.472 | 0.568 | 0.412 | 0.100 | 0.100 | 0.158 |
| 5,000 | 0.891 | 0.704 | 0.576 | 0.100 | 0.0 | 0.305 |
| 10,000 | 1.108 | 1.016 | 0.644 | 0.100 | 0.105 | 0.298 |
| 20,000 | 1.203 | 1.571 | 1.235 | 0.150 | 0.252 | 0.252 |
| Control | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

[a] Refers to group of 1st and 2nd instars; Group 2 consists of 3rd and 4th instars; Group 3 refers to 5th and 6th instars.

* Differ significantly from control at $p < .005$; $F = 38.3$ on (4,8) degrees of freedom.

** None of the treatments differ significantly from the control ($p < .05$), $F = 3.82$ on (4,8) degrees of freedom.

An alternative might be to utilize infective juveniles in some kind of trap where moisture could be controlled. A cockroach visiting the trap could conceivably pick up a number of infective juveniles, and transport them back to a common harborage, where they might be transferred to other members of the cockroach population.

## III. THE FUNCTION OF COCKROACH AGGREGATION: AN ASSESSMENT OF INDIVIDUAL INTERESTS

### Introduction

Reports of aggregation pheromones have appeared frequently in recent insect literature. These substances are defined by their ability to attract individuals of the species producing them without regard to sex or age, a property which has made them attractive for control oriented research. The German cockroach, *Blattella germanica*, has displayed such aggregation to feces and exudations of conspecifics, and it was suggested that a pheromone mediated this response. This result stimulated research on the pheromone's composition, mode of action, specificity, and site of production. Results of these studies indicate that the pheromone is composed of innumerable compounds, that it is perceived by touch and causes individuals that encounter it to stop, that it may attract other less frequent species, that glands near the anus are probably responsible for its production.

In the case of an aggregation pheromone, it is implied that individuals are providing information to which others are responding by aggregating, and that such a system will evolve only when the results benefit both the presumed signaller and respondent. Demonstrations of pheromone activity often come from responses only, which really only demonstrate that there is information to be had. An argument over whether or not communication is taking place is a semantic battle. Rather, it is more important to understand the interests of the parties involved.

Conditions that favor aggregation:

Aggregation should occur when individuals attain greater fitness by remaining in a group than they would if they were alone. This may occur when the group itself promotes individual survival, increases the frequency with which an individual will

mate, or when survivorship is promoted because individuals gather around a local abundance of resources. One accepted explanation for the aggregation of the German cockroach, is that roaches grow more rapidly in a group due to the group's amelioration of the local environment. Juvenile survival and adult weight have been shown to decrease with increased group size. The conclusion of rapid growth was a misinterpretation of data showing a reduced age of maturity. Data have been recorded for groups of 1, 2, 4, 8, and 16 individuals. Trends in survival and weight gain would tend to exclude the possibility of an ameliorated environment, while age of maturity showed unusual responses to increased group size. Male age of maturity plummetted when group size increased from 1 to 2, and remained similar for all groups. Female age of maturity was reduced more gradually with increasing group size.

Another condition favoring aggregation due to group properties occurs when grouping helps avoid predation. Scattering of the group may conter fitness by remaining in a group than they would if they were alone. This may occur when the group itself promotes individual survival, increases the frequency with which an individual will mate, or when survivorship is promoted because individuals gather around a local abundance of resources. One accepted explanation for the aggregation of the German cockroach, is that roaches grow more rapidly in a group due to the group's amelioration of the local environment. Juvenile survival and adult weight have been shown to decrease with increased group size. The conclusion of rapid growth was a misinterpretation of data showing a reduced age of maturity. Data have been recorded for groups of 1, 2, 4, 8, and 16 individuals. Trends in survival and weight gain would tend to exclude the possibility of an ameliorated environment, while age of maturity showed unusual responses to increased group size. Male age of maturity plummetted when group size increased from 1 to 2, and remained similar for all groups. Female age of maturity was reduced more gradually with increasing group size.

Another condition favoring aggregation due to group properties occurs when grouping helps avoid predation. Scattering of the group may confuse predators, which would benefit all group members. Predators are comparatively rare for domestic roaches, though mortality due to the efforts of man and his pets may be of some influence. There have been no studies of the forces of mortality operating within populations of domestic roaches.

There are two other possible explanations for aggregation which do not emphasize the role of the group in promoting survival and growth. The first is that individuals aggregate for purposes of mating. Those already in a group may have an interest in attracting the opposite sex to the group but may be powerless to stop others of the same sex from responding (and having access to those possible mates). This may be referred to as the eavesdropping hypothesis, and has been posed as an explanation for aggregation in crickets. This would explain aggregation without regard to sex, but does not explain aggregation among juveniles.

The remaining explanation differs in that information is not actively provided by group members. Those in a group may lose fitness due to competition for every individual attracted, but may be unable to hide the presence of the group. Outside the group, individuals may be making choices concerning habitats in which to settle based on the presence of conspecifics. A positive choice may be due to the presence of possible mates in the group, if this can be distinguished in the residues of the group members, or simply because the habitat has been tested (shown to be a safe or profitable one), which is demonstrated by the presence of conspecifics.

The purpose of this study was to collect data concerning these hypotheses on communication and aggregation for the German cockroach, Blattella germanica. The environmental amelioration hypothesis may be eliminated if group rearing does not affect or reduces survivorship and growth rate. The eavesdropping hypothesis may be eliminated if there is no sexual information present in the res##s compared with that of a strain resistant to most chlorinated hydrocarbons (collected from tenements in Baltimore, Maryland), since divergence for this characteristic should have occurred if finding a safe habitat has been important for the wild strain.

## Methods

Cultures of an industrial laboratory strain (CSMA-1) were obtained from the Raltech Corporation in Madison, Wisconsin. Cultures of a pesticide resistant strain from Baltimore, Maryland were obtained from Dr. E. Wood of the Entomology Department, University of Maryland, College Park, Maryland. Mass rearing was conducted under a regime of 24 hours per day of light. Temperature was maintained at 28°C, $\pm$ 2°C, with a relative humidity of > 50%.

Rearing experiments included 4 treatments: group sizes of 1, 2, 5, and 10 individuals per cage, all first instar on the day that groups were established. Food and water were constantly supplied. Replicates were of 20 individuals, with 20 replicates per treatment, for a total of 400 individuals undergoing each treatment. Replicates of each treatment were started on several different days, and treatments were equally dispersed throughout the space of the rearing facility. For each treatment, the number of individuals reaching adulthood and age of maturity were recorded. Weight upon reaching the last instar (adult) was measured for random subsample from each treatment.

Bioassays recorded the responses of lone individuals, so as not to confuse responses to treatments with responses to other individuals. Experiments relied on a shelter seeking response. Three small paper shelters (1.5 cm x 4.0 cm papers folded into an upside down "V") were placed inside 150 mm x 20 mm glass petri dishes (cleaned before each use), at equal distances around the periphery of the dishes, and with food and water available at the center of the dish. Shelters were cut from 1.0 m x 0.1 m segments of paper that were corrugated and exposed to roach populations for three days. Populations used in paper treatment were either 40 adult males, 40 virgin adult females, or 80 third and fourth instar juveniles of mixed sex. Tests were run for 48 hours, after which the number of fecal pellets under each shelter was tallied. The proportion of total fecal pellets in each dish was used to rank responses to each shelter type. The experiment was performed 60 times for each instar from third through adult (30 times for males and 30 times for females). Orientation of shelters within each series was varied so that all possible permutations were tried with equal frequency.

To compare the laboratory strain with the resistant strain from Baltimore, a similar experiment was performed. Paper was treated as before, using a general population of 100 individuals for 3 days. Two shelters were placed in each dish, one treated while the other was not. Left and right orientations for the treated shelter were equally frequent. Thirty experiments were conducted using the fourth instar (male or female) of each strain, for a total of 60 experiments. Since comparisons were never made within strains, ranks were not needed, and comparisons were made for proportions.

## Results

The weight reached at adulthood showed no significant change as group size increased (Figure 1). Daily survival rates (DSR) were calculated as the geometric mean of juvenile survival for the average age of maturity for the treatment. The relation between DSR and group size is illustrated in Figure 2. Total juvenile survival differed by less than 0.5 percent for group sizes of 1 and 2. (Male and female differences are exaggerated by the figure.)

Results of assays which determine the presence or absence of sexual information in residues are displayed in Figures 3 and 4. The ordinate is the average proportion of responses of each of male, female, and juvenile residues. Within series of experiments done for each sex and instar, the ranks generated for the proportions from each experiment were used in a Friedman nonparametric two-way analysis of variance, and pairwise least significant difference (LSD) tests. For males (Figure 3), responses to female residues appear to increase steadily with age. A one-way ANOVA of these proportions of responses directed to the female stations among the various instars tested was significant ($P \leq 0.01$). Females do display some signficant differences in response, but responses do not appear to change with age in any distinct progression (Figure 4). Response to juveniles was highest at adulthood, and variation among instars for this response to juveniles was significant ($P \leq 0.04$).

Responses of CSMA and Baltimore strains differed when given a choice of residues or nothing (Table 1). Response to residues was greater for the Baltimore strain, with borderline significance. One sample comparisons with an expected proportion of responses in favor of residues of 0.5 was not significant for the CSMA strain, but approached significance for the Baltimore strain ($P \leq 0.06$).

Table 1. A Comparison of responses by CSMA and Baltimore strains to residues of a general population.

| Strain | CSMA | Baltimore | |
|---|---|---|---|
| $\bar{x}$ | 0.4670 | 0.5976 | $t = 1.428$ |
| $s_x$ | 0.3660 | 0.3142 | $P \leq 0.10$ |
| N | 27 | 29 | |

## Discussion

Results of the rearing experiments eliminate the environmental amelioration hypothesis. Though the reduction in growth was not significant, there was clearly no increase as suggested in previous literature. It is possible that rearing in small sealed containers ameliorated the environment in the same way that grouping might have so that only the effects of competition could be observed as group size increased. Such experiments should be repeated with other treatments increasing exposure in order to establish that the containers were not responsible for this result. If containers could be contrived so as to provide greater exposure to the outside environment, amelioration may yet be seen. This was not done as a part of this study due to space constraints.

Field studies of the forces of mortality operating within natural populations of these insects would be important in establishing or eliminating the influence of grouping on survivorship. The literature asserts that predators of domestic roaches are rare or nonexistent. The consideration omitted the possibility of predation by rats, mice, housepets, or the facsimile of predation often delivered by man's shoes. Group scattering could confuse these predators and increase individual survivorship. These pressures could result in divergence among populations, which unfortunately would appear much the same as the divergence that should result under the habitat selection hypothesis.

A primary consideration in the elimination of the predator confusion hypothesis of whether or not aggregates occur in locations with high potential for predation. Observations (personal) suggest that these aggregates occur primarily in locations such as crevices where predators could not normally reach them. One goal for further field studies on roach mortality would be to answer this question of where aggregates occur. If they may occur outside of crevices, then elimination of the predator confusion hypothesis will come from a demonstration that per capita mortality due to predation is equal with or without grouping.

Information about the sexual composition of a population must be present in residues of that population in order for the eavesdropping hypothesis to operate. Males displayed a much stronger response to the opposite sex than did females, with an interest in the sexual composition of the population which steadily increases with age. Intuitively, the eavesdropping explanation works best when males are attempting to draw females, since a male's fitness increases for each female to which he has access. Females may increase their fitness by increasing the range of mates from which they may choose, but returns should diminish quickly since one very fit male is as good as another. Since males seem to be responding to females rather than females to males, this might be construed as evidence against the eavesdropping hypothesis. An enlightening series of experiments in this regard would entail conducting the three choice bioassay again for adults, removing shelters treated by the opposite sex, and replacing them with untreated shelters. Choices would have to be made between individuals of the same sex, construed for argument as displaying, and young juveniles.

That virgin females appear not to be attracted to adult males is unusual. Females that remain uninseminated after their first week of adulthood continue to mature eggs, though do not complete construction of an ootheca. This clutch is wasted, an apparent loss of fitness for these females. One possibility is that a female in need of a mate should hold still for males which are searching for her. Males are drawn to areas marked by females, so that females which stay in areas marked by many males may go unfound.

## IV. BIBLIOGRAPHY OF PUBLICATIONS PREPARED UNDER THIS CONTRACT

### Publications

Koenig, J. P. 1982. Susceptibility of the German cockroach, Blattella germanica, to Neoaplectanid Nematodes. M. Sc. thesis, University of Wisconsin-Madison.

Wileyto, E. P. and G. M. Boush. 1983. Attraction of the German cockroach, Blattella germanica (Orthoptera: Blatellidae), to some volatile food components. J. Econ. Entomol. 76: 752-756.

Wileyto, E. P. and G. M. Boush. 1983. A new method for individually rearing the German cockroach (Orthoptera: Blattellidae). J. Econ. Entomol. 76: 676-677.

Manuscripts

Boush, G. M., E. P. Wileyto, and D. A. Ehrhardt. ____. Cockroach control: A search for new answers to an old problem. Submitted 12/6/82 to Naval Research Reviews.

Hassan, H. F. 1983. The role of the symbiotic bacterium, Xenorhabdus nematophilus in mass-rearing of Neoaplectanid Nematodes, M. Sc. thesis, University of Wisconsin-Madison (to be presented in September,1983).

Wileyto, E. P., G. M. Boush, and L. M. Garvin. ____. The function of cockroach aggregation; an assessment of individual interests. Environ. Entomol. (Submitted 8/22/83 for publication.)

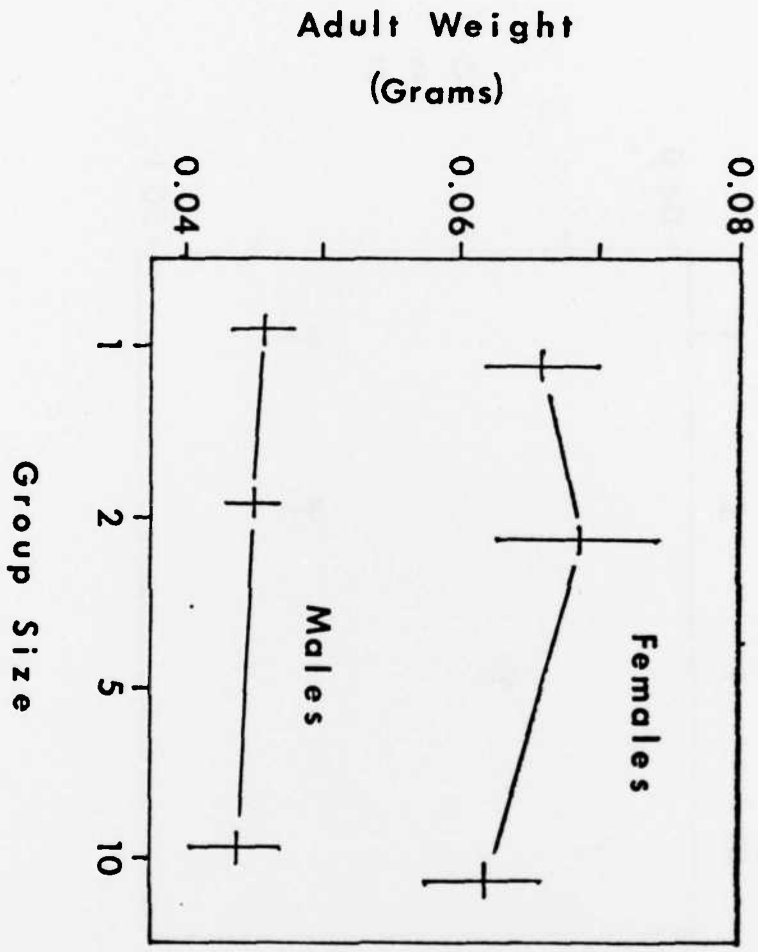


Figure 1. Weight at maturity for roaches reared in various group sizes.

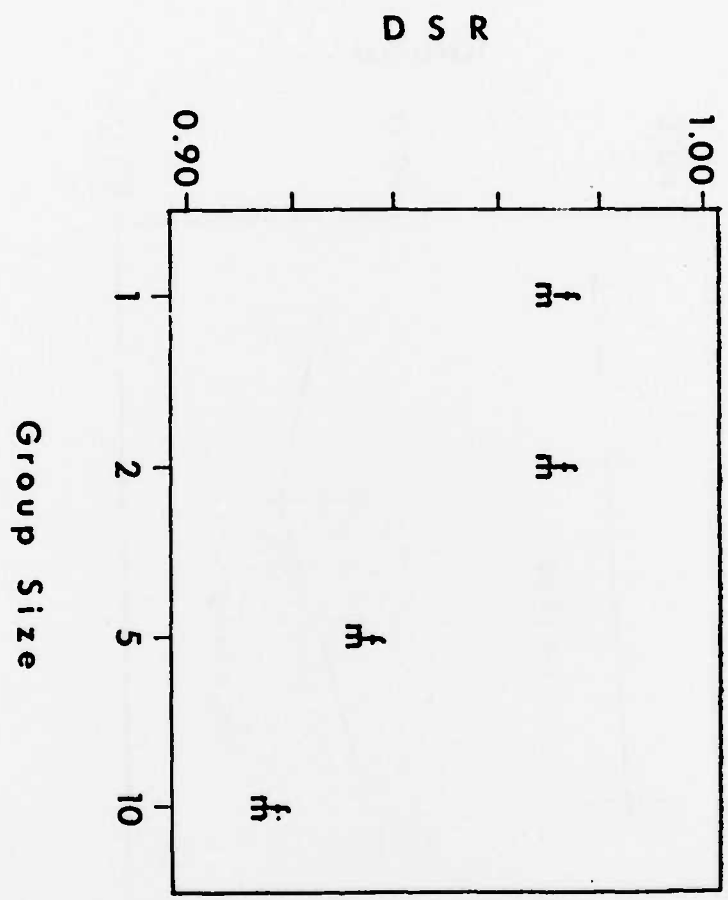


Figure 2. Daily survival rate estimates (DSR) for individuals reared under differing group sizes. (m = male, f = female). Male-female differences are exaggerated by this figure.

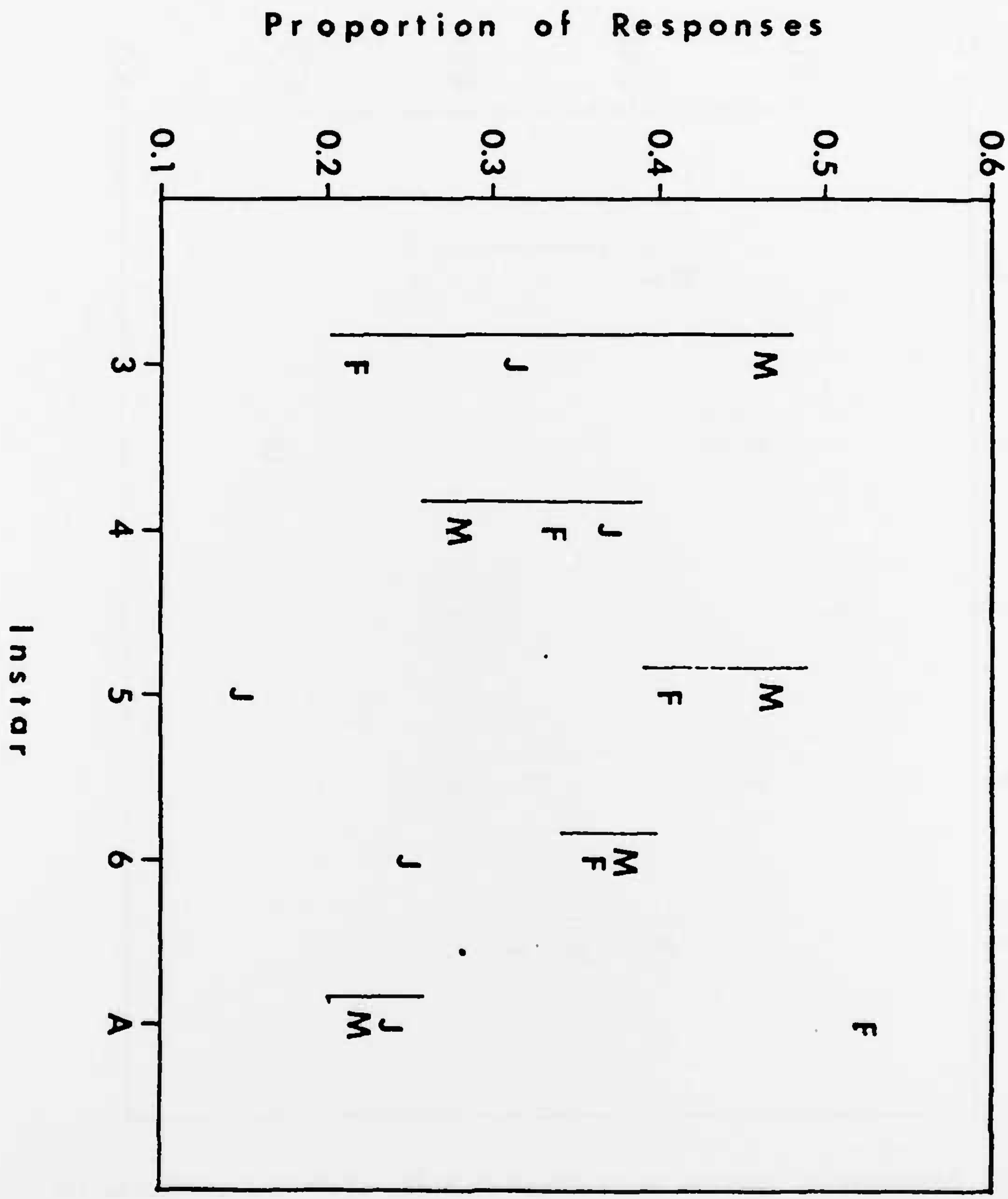


Figure 3. Responses of males to residues of males, females, and juveniles (M, F, and J respectively). Bars indicate responses that are not different ($P \leq 0.05$) by Friedman nonparametric ANOVA and LSD tests.

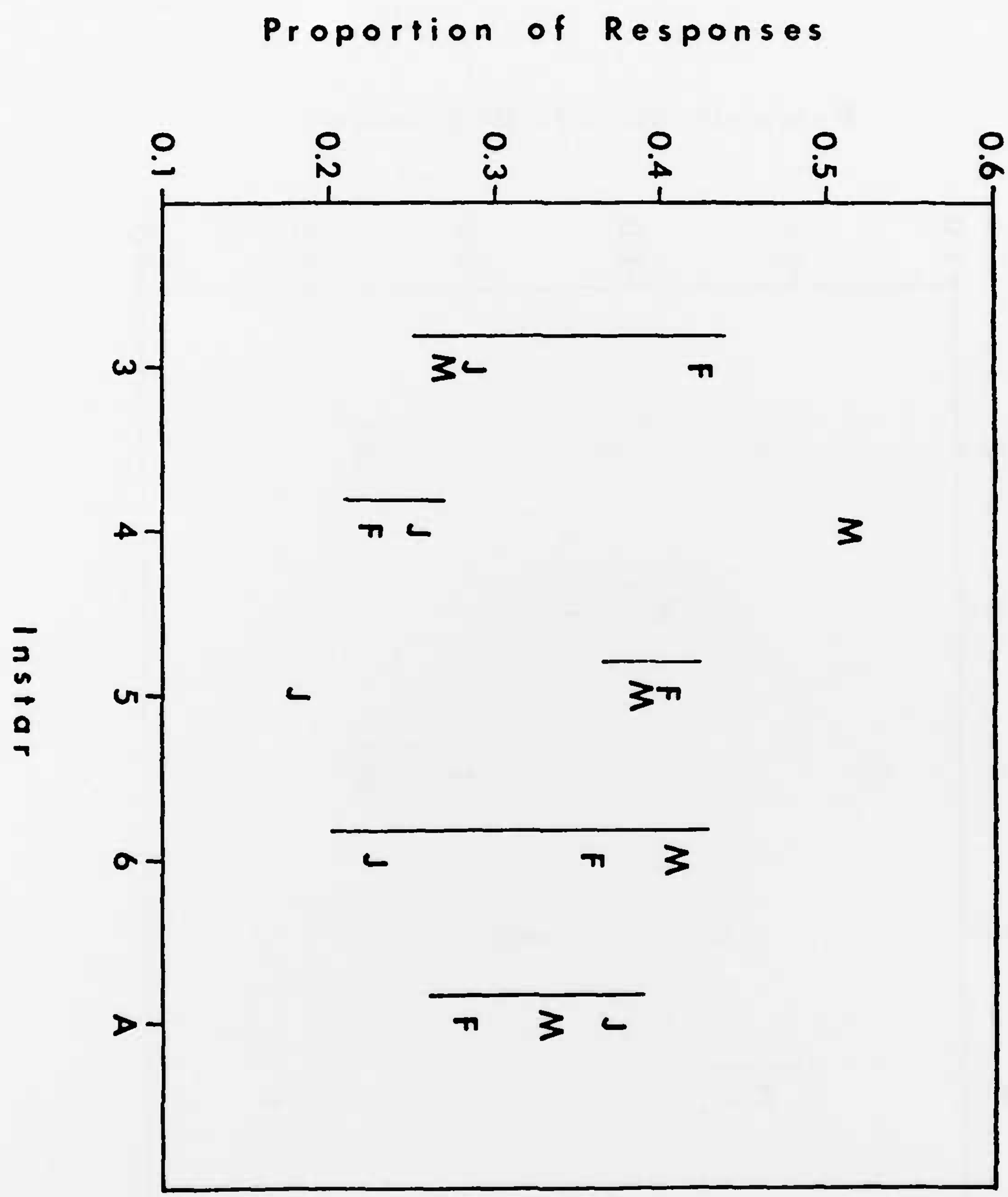


Figure 4. Responses of females to residues of males, females, and juveniles (M, F, and J respectively). Bars indicate responses that are not different ($P \leq 0.05$) by Friedman nonparametric ANOVA and LSD tests.

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